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DNA vaccination strategies against infectious diseases

Allison M. Watts, Ronald C. Kennedy *

Department of Microbiology and Immunology, The University of Oklahoma Health Science Center, 800 North Research Parkway, Suite 462, Oklahoma City, OK 73104, USA

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Abstract

DNA immunisation represents a novel approach to vaccine and immunotherapeutic development. Injection of plasmid DNA encoding a foreign gene of interest can result in the subsequent expression of the foreign gene products and the induction of an immune response within a host. This is relevant to prophylactic and therapeutic vaccination strategies when the foreign gene represents a protective epitope from a pathogen. The recent demonstration by a number of laboratories that these immune responses evoke protective immunity against some infectious diseases and cancers provides support for the use of this approach. In this article, we attempt to present an informative and unbiased representation of the field of DNA immunisation. The focus is on studies that impart information on the development of vaccination strategies against a number of human and animal pathogens. Investigations that describe the mechanism(s) of protective immunity induced by DNA immunisation highlight the advantages and disadvantages of this approach to developing vaccines within a given system. A variety of systems in which DNA vaccination has resulted in the induction of protective immunity, as well as the correlates associated with these protective immune responses, will be described. Particular attention will focus on systems involving parasitic diseases. Finally, the potential of DNA immunisation is discussed as it relates to veterinary medicine and its role as a possible vaccine strategy against animal coccidioses.

Keywords: Animal coccidioses; DNA immunisation; DNA vaccines; Parasitic infections

1. Historical perspectives of nucleic acid immunisation

Concepts related to gene delivery for therapeutic modalities began with the DNA transfer experiments that were initiated and described in the 1950s. In these studies, crude DNA preparations isolated from neoplastic tumours were shown to induce in vivo tumour formation when inoculated into rodents. Experiments decades later demonstrated that the direct injection of DNA without any contaminating material resulted in the in vivo expression of DNA encoded gene products. The potential for DNA immunisation as a means of inducing an antigen-specific immune response had become apparent (reviewed in [1]).

The ability of DNA to induce an immune response in vivo may have first been demonstrated in 1962 [2]. In these investigations, the

* Corresponding author. Tel.: +1-405-271-5630; fax: +1-405-271-6339; e-mail: ronald-kennedy@ouhsc.edu

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s.c. inoculation of crude preparations of polyomavirus DNA containing transforming sequences into newborn hamsters resulted not only in the induction of tumours, but also in the production of antibodies specific to the virus. However, it was unclear whether the tumour induced by the DNA was responsible for the induction of the anti-viral antibodies or whether the DNA inoculation itself generated the immune response. Other investigators demonstrated that inoculation of polyomavirus DNA resulted in more hamsters developing anti-viral antibodies than tumours [3], suggesting that the DNA itself could induce an anti-viral specific immune response. These early observations were later confirmed by inoculation of mice and hamsters with recombinant forms of polyomavirus DNA [4]. Together, these studies suggested that immune responses may be induced as a result of DNA injection (reviewed in [1]).

In the early 1980s, two independent groups of investigators demonstrated that the expression of plasmids encoding hepatitis B viral proteins and insulin could induce an immune response when injected into animals [5,6]. However, the major turning point regarding the in vivo expression of a foreign gene inserted into a plasmid via direct DNA inoculation was reported in 1990 by Wolff et al. [7]. Utilising chloramphenicol acetyl transferase, luciferase, or β-galactosidase as the foreign reporter gene, these investigators demonstrated that inoculation of either purified RNA or DNA into the skeletal muscle resulted in expression of the enzyme. The detection of episomal DNA by Southern blot analysis 30 days p.i. and the presence of enzyme activity 60 days after injection provided evidence that the foreign reporter genes were being taken up and expressed in vivo. That the majority of the injected DNA persisted as extrachromosomal episomal DNA within the muscle may have potentially been the result of the low proliferative state of the myocytes and/or reflected the structural features of the muscle that make it particularly suited to uptake polynucleotides. However, the authors were not able to rule out the possibility that low levels of chromosomal integration of the injected DNA had occurred. It was not known whether these transcribed and translated gene products could be presented to the immune system and generate an immune response.

In 1992, Tang et al. [8] found that plasmids coated onto gold beads resulted in foreign gene expression and the induction of an antibody response to the foreign gene product in mice. They used genetic immunisation as a means of generating a humoral immune response to a gene product by injecting plasmid DNA encoding the foreign gene into a host. Following this report, a number of other investigators employed plasmid DNA immunisation to induce humoral and cell mediated immune responses to influenza A virus [9,10], human immunodeficiency virus (HIV) [11], and hepatitis B virus surface antigen [12], and collectively demonstrated the potential for DNA immunisation as a vaccine strategy against viral pathogens. The potential of DNA immunisation as a method for cancer immunotherapy was also reported [13–16]. Other early investigations also demonstrated the use of DNA immunisation for the induction of antibodies to immunoglobulin light chains [17]. Thus, it was clear that immunisation with DNA could generate antigen-specific immune responses to a specified gene product, and could therefore potentially serve as an immunotherapeutic modality.

2. Delivery of DNA vaccine

From a simplistic view, DNA vaccination requires that the plasmid DNA enters a cell, be transcribed and translated, and the foreign gene product be presented as an antigen in tissues accessible to the immune system. For successful transfection of the DNA and expression of the antigen, the plasmid should include an efficient promoter to drive transcription of the encoded antigen. A number of promoters are suitable to drive transcription. These can include sequences derived from genomic DNA which provide efficient autonomous replication in a variety of mammalian cells. Many plasmid DNA immunisation schemes to date employ strong viral promoters, one of the most efficient being the

human cytomegalovirus (CMV) immediate/early promoter. The optimal plasmid should be in a supercoiled state and contain a 3′ poly A tail to ensure the stability of the transcribed mRNA. Most plasmids also encode a poly A sequence such as the SV40 late poly-adenylation signal.

The site of inoculation plays an important role in the induction of protective immune responses to DNA vaccination. The most easily accessible tissues for DNA immunisation are the exterior skin and mucosal surfaces of a host. In studies evaluating route of delivery, DNA inoculation induced protective immune responses when administered mucosally to the nasal passages or trachea of experimental animals. A significantly higher level of protection was obtained when the DNA was delivered to the skin by methods that enhanced uptake of the DNA and increased the transfection efficiency [10]. Although cardiac muscle, liver, and dermis have been shown to express gene products after DNA inoculation, the optimal response is seen with inoculation into skeletal muscle. In direct comparative studies in mice, i.m., i.v., intranasal, intradermal, and s.c. routes of inoculation of DNA induced some protection against a lethal influenza virus experimental challenge [10], but the i.p. route did not. The i.m. and i.v. routes of inoculation resulted in the best responses within this system. In chickens, the i.m., i.v., and mucosal administration of DNA provided some protective immunity against a lethal challenge with avian influenza virus [10], with little to no protection being observed by s.c., i.p., intrabursal, and intraorbital routes of inoculation. Thus the route of DNA inoculation is important in the induction of protective immune responses, and the optimal route may vary with the system being examined.

The in vivo transfection efficiency of DNA has been enhanced by, for example, adsorption of DNA to gold particles and delivery through the use of a gene gun [8, 10] that bombards the skin with gold particles containing the plasmid DNA. The gold particles directly penetrate the skin due to the force of delivery, thereby increasing the rate of transfection without having to rely on the uptake of DNA by the host cell itself. At least 100-fold less plasmid DNA is required for the induction of protective immune responses when administered adsorbed to gold particles and delivered by a gene gun when compared with inoculation of plasmid DNA in saline using a syringe and needle; as little as 0.4 μg of DNA can induce protective immunity in mice when compared with hundreds of micrograms of DNA in saline administered without a gene gun [10]. Additional techniques utilised to enhance the in vivo uptake of inoculated DNA include the use of local anaesthetics, such as bupivacaine (1-butyl-*N*-(2,6-dimethylphenyl)-2-piperidinecarboxamide), myotoxins such as cardiotoxins, and a variety of ionic and lipid-based delivery systems (reviewed in [1]). However, in a number of systems the use of saline alone as a carrier moiety for DNA immunisation has resulted in the induction of specific immune responses and appears to be a reasonable approach if large quantities of plasmid DNA are available (reviewed in [1]). It is anticipated that studies on the most effective way to deliver plasmid DNA to the immune system for the induction of an immune response will continue to evolve and new delivery systems and vehicles will be evaluated.

3. Presumed mechanisms for DNA immunisation and immune responses

Cell-mediated immune responses can be divided into major histocompatibility complex (MHC) class I- or class II-specific. The MHC class I pathway presents endogenously synthesised protein antigens that are initially translated on the ribosomes of the endoplasmic reticulum (ER) or cytosol. The proteins are then processed into peptides and associated with MHC class I molecules in the lumen of the ER. A variety of other accessory molecules, including the transporter proteins TAP-1 and TAP-2, assist in the targeting and association of the peptide antigen with the MHC class I molecules (Fig. 1). The peptide–MHC class I complex is transported to the cell surface where it is recognised by the T-cell receptor (TCR) expressed on $CD8^+$ T cells. Activated $CD8^+$ cytotoxic T lymphocytes (CTL) then target MHC class I antigen present-

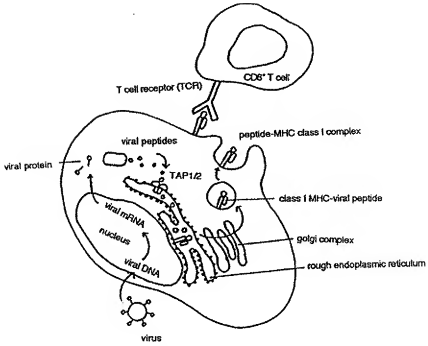

Fig. 1. In the MHC class I pathway of antigen presentation, endogenously synthesised proteins (such as products of viral transcription/translation) are processed into peptides in the cytoplasm and are then actively transported into the lumen of the rough endoplasmic reticulum by TAP 1/2 transport proteins. Within the lumen, peptide associates with the MHC class I molecule. The complex is transported out through the Golgi complex to the cell surface where it is then recognised by the $CD8^+$ T-cell receptor. Recognition leads to activation of a cell-mediated CTL immune response specific for the antigen presented.

ing cells and subsequently lyse them. Also associated with MHC class I-restricted responses are $CD8^+$ T cells with suppressor activity. When activated, these cells can induce a state of unresponsiveness and/or anergy. With regard to vaccination, the induction of CTL responses is important when the organism in question is an intracellular pathogen. Activation of the endogenous pathway and the induction of MHC class I-restricted responses can result from infection of a cell by a pathogen, an attenuated vaccine that replicates in host cells, or a replicating vector that infects the cell prior to synthesis of the inserted foreign protein.

In contrast to the MHC class I pathway, the MHC class II pathway presents antigens that are processed exogenously. Extracellular proteins are acquired by endocytosis and are processed in endosomes into antigenic peptides that associate with MHC class II molecules. The peptide-MHC class II complex is transported to the cell surface where it is recognised by the T-cell receptor (TCR) expressed on $CD4^+$ T cells. The $CD4^+$ T cell becomes activated with the aid of a number of co-stimulatory molecules on the surfaces of both $CD4^+$ T cells and MHC class II antigen presenting cells. A $CD4^+$ T cell may then act as a T-helper lymphocyte and activate antigen-specific B cells to secrete antibody (Fig. 2). Inactivated and protein subunit component vaccines are predominantly presented by MHC class II pathways. The induction of MHC class II pathways by a particular vaccination strategy is appropriate when the pathogen is extracellular. Such is the case with cell-free virus or bacteria where neutralising or opsonising antibodies mediate protective immunity.

The $CD4^+$ T-cell population has been subdivided into T_H1 and T_H2 subsets based on the profile of cytokines secreted following activation.

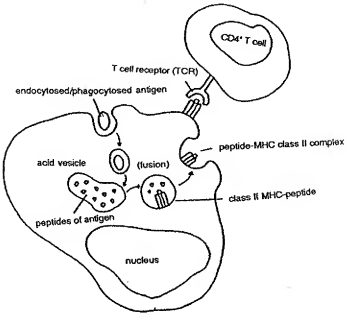

Fig. 2. In the MHC class II pathway of antigen presentation, endocytosed/phagocytosed protein antigens are processed into peptides within acidic endosomes. The endosomes then fuse with vesicles containing the MHC class II molecule, resulting in MHC class II–peptide complex formation. The complex is transported to the cell surface, where it is recognised by the $CD4^+$ T-cell receptor. Recognition leads to the activation of T helper lymphocytes which may produce an inflammatory reponse (T_H1), or antibody secretion by B cells (T_H2).

Among the cytokines secreted by the T_H1 $CD4^+$ cells are interferon-gamma (IFN-γ) and interleukin-2 (IL-2). Secretion of these cytokines results in T-cell proliferation and upregulation of MHC class II expression. The T_H1 $CD4^+$ cells seem to contribute only limited help for B cells in antibody secretion, yet they provide potent cell-mediated immune responses by stimulating CTL and increasing the phagocytic activity of monocytes and macrophages. Alternatively, the T_H2 $CD4^+$ T-cell population secretes cytokines such as IL-4, IL-5, IL-6, and IL-10 which cause the activation of B cells and induction of antibody isotype switch and secretion. These cells play a major role in supplying B cell help in the generation of a humoral immune response.

Other cytokines produced by T cells can modulate the immune response by directing it to either the T_H1 or T_H2 pathway; an outcome of direct manipulation of the immune response by co-injection of cytokine genes in association with DNA immunisation [18]. The co-delivery of IL-12 with a plasmid encoding HIV gp160 induced splenomegaly in mice, along with a reduction in antibody responses, and a dramatic increase in CTL activity. From this observation it was concluded that IL-12 suppresses T_H2 pathways and stimulates T_H1 responses. Co-delivery of granulocyte–macrophage colony stimulation factor (GM-CSF) resulted in an increase in antibody responses (activates T_H2) and did not appear to enhance CTL activity [19]. Therefore, it has become clear that the immune response generated by immunisation can be directed to a humoral or an inflammatory response with the co-administration of select cytokines.

4. Plasmid DNA immunisation and immunostimulatory properties

Studies based on antibody isotypes and cytokine secretion profiles have demonstrated that plasmid DNA immunisation primes T_H1

responses [20]. Mice injected with DNA encoding β-galactosidase showed IgG2a antibody isotype secretion and $CD4^+$ T-cell secretion of IFN-γ, but not IL-4 and IL-5. In contrast, mice given protein in saline or alum produced IgG1 and IgE β-galactosidase-specific antibodies and $CD4^+$ T-cell secretion of IL-4 and IL-5, but not IFN-γ. It was concluded that DNA immunisation induced a predominant T_H1 response, whereas protein immunisation induced a T_H2 response to the same antigen. Subsequently, short immunostimulatory sequences (ISS) were identified within the plasmid DNA sequence that enhanced the immunogenicity of the plasmid [21]. The ISS contain a CxG dinucleotide sequence in a particular base context, and this has also been referred to as the CpG immunostimulatory motif. Human monocytes transfected with plasmid DNA or double-stranded oligonucleotides containing the ISS transcribed large amounts of IFN-α, IFN-β, and IL-12 [21]. The ISS are not necessary for DNA immunisation to evoke antigen-specific immune responses, but they may alter or interfere with a particular immune response by inducing proinflammatory cytokines that can modulate immunologic pathways involved in protective immunity. However, it remains to be determined whether ISS exclusively target T_H1 pathways.

5. Studies involving DNA vaccines against human pathogens

Many DNA vaccines that target viral pathogens (reviewed in [1,22]) have been examined. Influenza virus was one of the first DNA immunisation experimental models to be evaluated. The specific antigens employed to target the immune response against influenza virus are the viral haemagglutinin (HA) and viral nucleoprotein (NP). Inoculation (i.m.) of mice with plasmid encoding the HA and NP genes induced HA inhibiting antibodies and CTL responses [23] and protection from experimental influenza virus challenge correlated with the amount of DNA injected and the titres of HA inhibiting antibody. Immunogenicity studies with nonhuman primates also revealed induction of HA inhibiting antibodies, though at lower levels than reported in mice [24].

Immunisation with plasmid DNA encoding HIV-1 gp160 induced humoral and cell-mediated immune responses in mice and subsequently, in cynomolgus monkeys (*Macaca fascicularis*) [25]. Both humoral and cell-mediated immune responses to HIV-1 gp160 were observed. The cynomolgus monkeys were challenged with a SHIV chimeric virus expressing the HIV-1 envelope gene encoding gp160, the simian immunodeficiency virus (SIV) core, and other structural and regulatory genes. Of the four DNA vaccinated and SHIV challenged monkeys, one developed a viral load similar to control animals, two developed transient viraemia and cleared the infection within 60 days, and one was completely protected. To evaluate the protective capacity of DNA vaccines against an experimental challenge with HIV-1, chimpanzees were immunised with a DNA vaccine preparation encoding the *env*, *rev*, and *gag/pol* genes from HIV-1 [26]. The immunised chimpanzees developed HIV-1 specific humoral and cell-mediated immune responses. Chimpanzees were challenged with a heterologous isolate of HIV-1 and, although both DNA vaccinated chimpanzees were protected from the establishment of infection, a transient viraemia was observed when compared with a single control infected animal. These studies implicate the protective potential of DNA vaccination strategies in a nonhuman primate model of experimental infection with HIV-1.

The potential of DNA vaccination as an immunotherapeutic modality in experimentally infected chimpanzees was also evaluated [27]. One HIV-1 infected chimpanzee was inoculated with plasmid DNA encoding the *env* structural and *rev* regulatory gene and a second was inoculated with a control plasmid DNA. The therapeutically HIV-1 DNA vaccinated chimpanzee demonstrated an enhanced humoral immune response and a decrease in viral load at week 20 following plasmid DNA injection, and remained at background levels during the course of the study. Although the therapeutic effectiveness could not be determined with such a small sample size, the authors concluded that the

immune response can have a direct impact on HIV-1 replication in chimpanzees.

Other investigators evaluated a DNA vaccination strategy within the SIV system. The SIV–macaque monkey model was utilised because it has many similarities with HIV-1 infection in humans and the sequelae to AIDS. Immunisation of macaques with plasmid DNA encoding either the SIV *env* gene receptor binding site or the extracellular domains of the *env* gene resulted in the induction of specific antibodies and $CD8^+$ MHC class I restricted CTL [28], although the animals were not protected from an experimental SIV challenge. Although these two sets of studies were contradictory with regard to the induction of protective immunity within the HIV-1/SIV primate model systems, it should be pointed out that the plasmid DNA constructs used in the HIV-1 and SIV studies were different. Additionally, the experimental challenge systems employed different monkeys and various HIV/SIV infectious inocula to evaluate protection from infection and/or disease. It remains to be determined whether DNA encoding HIV gene products will evoke protective immunity and prevent AIDS in humans.

Herpes viruses are a significant human pathogen and, despite decades of studies and years of evaluation, no commercial licensed vaccine is available. A repeatedly observed problem with the development of a vaccine against herpes simplex virus (HSV) in human clinical trials is that placebo vaccination may give up to 35% protection. Herpes simplex virus also represents a human pathogen where both prophylactic and post-exposure therapeutic vaccination strategies are appropriate. At least four groups of investigators have described the induction of HSV-specific immune responses and protection in animal models following injection with DNA encoding HSV proteins [29–32]. DNA immunisation with plasmids encoding a HSV-1 regulatory protein, designated ICP27 or the structural gene for the major glycoprotein gB, generated an immune response that resembled for the most part the immune response following exposure to replicating virus in mice [29, 33]. Immunisation with gB encoding plasmid DNA induced a neutralising antibody response where the IgG2a isotype was dominant. The $CD4^+$ T-cell response had predominantly a T_H1 cytokine profile, but a CTL response was not detected, suggesting that a T_H1 cytokine profile is not always associated with a CTL-specific cell-mediated immune response. ICP27 DNA immunisation resulted in the induction of CTL response, but no specific antibodies were detected. To evaluate protective immunity, a murine model (zosteriform model) which results in the development of cutaneous lesions following HSV-1 challenge was employed. The cutaneous lesions are the result of viral dissemination along sensory nerves following replication in the nerve ganglion. Protection following either ICP27 DNA or gB DNA immunisation was observed in mice challenged with a low dose of HSV-1, but was not observed with a 50-fold higher challenge inoculum. In contrast, animals immunised with either an infectious virus or recombinant vaccinia virus expressing the HSV proteins were solidly immune to both the high and low dose HSV-1 challenge (reviewed in [34]). Protective immunity correlated with $CD4^+$ T cells as protein could be transferred adoptively in vivo. These data indicate that an attenuated vaccine or recombinant replicating vector strategy induce more complete immunity in an animal model system compared with DNA approaches.

The human microbial pathogen *Mycobacterium tuberculosis* is also a subject of DNA vaccination strategies. Even with antibacterial drug treatment and vaccination with an attenuated *Mycobacterium bovis* variant, bacille Calmette–Guerin (BCG), it has been estimated that there are still 10 million new cases of tuberculosis (TB) every year [35, 36]. Tuberculosis occurs primarily in developing countries and results in 3 million deaths worldwide each year. With the development of multi-drug-resistant strains, and the ineffectiveness of the BCG vaccine in inducing protective immunity, DNA vaccination strategies are being actively examined. Immunisation with plasmid DNA encoding a secreted component of *M. tuberculosis*, designated antigen 85, generates humoral and cell-mediated immune responses in mice [35]. DNA vaccines that encode the *Mycobacterium leprae* heat shock protein, hsp65,

which is highly conserved among *Mycobacteria* species and appears antigenically similar to that of *M. tuberculosis*, has given similar results in mice [36]. Both DNA vaccines induced protective immunity in mice following experimental challenge with live *Mycobacterium*, and although protection induced by the hsp65 DNA vaccine was comparable with that arising from BCG immunisation, immunising with the protein alone failed to generate protective immunity against infectious challenge. Studies involving the use of DNA vaccines against a variety of microbial pathogens have highlighted the success and failures of this approach for inducing protective immunity in comparison with more conventional vaccine approaches (reviewed in [34]). It is apparent that in a number of situations DNA vaccination strategies require further refinement to improve immunogenicity and the induction of protective immunity.

6. Studies of DNA vaccines involving parasitic diseases

DNA immunisation against the malaria parasite has been extensively studied. The potential efficacy of a DNA vaccination strategy utilised the *Plasmodium yoelii* rodent malaria model [37], and mice were immunised i.m. with one of two different plasmids containing the *P. yoelii* circumsporozoite (CSP) gene. Both humoral and cell-mediated immune responses were induced after challenge with a high dose of sporozoites. A significant reduction in the number of liver stage schizonts was found in DNA vaccinated mice compared with mice given irradiated sporozoites. With a low challenge dose, sterile immunity was observed in some of the DNA immunised mice. The correlate of immunity was shown to be associated with $CD8^+$ T-cells as treatment of immunised and protected mice with a monoclonal anti-CD8 completely abolished protective immunity and $CD8^+$ CTL were probably responsible for eliminating *Plasmodium* infected hepatocytes. Encouragingly, inoculation of DNA containing genes from different parasite stages can bypass the genetic restriction of the immune response in mice that was observed with protein immunisation [38]. $CD8^+$ T cells, IFN-γ, and nitric oxide were also found to play a role in the protective immunity against malaria in mice. More recently, a mixture of four DNA plasmids encoding multiple antigens from *Plasmodium falciparum* was used to immunise rhesus monkeys (*Macaca mulatta*) [39]. The majority of immunised monkeys given either the individual plasmids or a combination of plasmids generated antigen-specific CTL, providing a foundation for the evaluation of multigene based immunisations to protect against malaria in humans. Immunisation with DNA plasmid encoding the CSP gene from *Plasmodium berghei* and expressing different amounts of the CSP were given by various routes of inoculation [40]. The strongest humoral immune response and the greatest level of protection was induced by epidermal vaccination with the high level CSP expressing plasmid. The immunisation protocol also utilised a gene gun to deliver three immunisations at 6 week intervals. The predominant antibody response to CSP was the IgG2a subclass suggestive of a T_H1 response and the authors concluded that the interval dependent induction of these antibodies by epidermal immunisation contradicted the concept that antibody responses induced by this method result in a T_H2-dependent response.

The enhanced immunogenicity for $CD8^+$ T-cell induction and protective immunity in mice primed with DNA immunisation (encoded pre-erythrocytic antigens from *P. berghei*) and boosted with a vaccinia virus vector (MVA) expressing the same malarial antigen has been described [41]. Thus DNA immunisation can be utilised with other vaccination modalities to enhance the immune response when compared with that generated by a single vaccination strategy alone. Such a prime-boost strategy was highly effective at inducing protective immunity associated with $CD8^+$ T cells in mice. It was of interest to note that when the immunisation was reversed, no protective immunity was observed.

The use of somatic transgene immunisation (STI) has also recently been described to provide immunity against *P. falciparum* malaria sporo-

zoites in mice [42]. Somatic transgene immunisation is an alternative approach to DNA based vaccination and is induced with transgenes under the control of lymphoid tissue specific regulatory elements. This approach requires direct inoculation of lymphoid tissues, such as the spleen, to allow long-term expression of the transgene in vivo. This represents a new modification of DNA immunisation technology that requires further evaluation to determine its effectiveness as an immunisation vehicle for vaccine delivery.

Schistosomes are trematode parasites of mammalian species that infect approximately 200 million people worldwide. The efficacy of nucleic acid immunisation for inducing protective immune responses against *Schistosoma japonicum*, the Asian blood fluke, has been evaluated in murine models. Mice were immunised with a variety of plasmids encoding a number of different gene products produced by schistosomes. These genes included the glutathione-*S*-transferase (GST), calreticulin, glyceraldehyde-3-phosphate dehydrogenase, an unidentified membrane associated antigen, a 14 kDa fatty-acid binding protein, fragments of paramyosin, full-length paramyosin, and a fusion between paramyosin and GST [43]. The paramyosin encoding DNA plasmids all induced antibodies to anti-paramyosin, and the paramyosin–GST gene fusion. None of the other DNA plasmids induced detectable antibody responses. The anti-paramyosin antibodies failed to protect mice from a challenge with *S. japonicum* cercariae.

Other investigations have described DNA immunisation to induce specific antibody responses to the 28 kDa GST protective antigen from *Schistosoma mansoni*. Three injections of 200 μg of plasmid DNA encoding the 28 kDa GST at 14 day intervals into the skin of rats resulted in production of IgG antibodies [44]. Sera from the immunised rats mediated antibody-dependent cell-mediated cytotoxicity in vitro with specific killing of parasite larvae. Challenge with *S. mansoni* infectious cercariae resulted in a boosting of the IgG response in DNA immunised animals, although protection against infection was not observed. Investigations have also shown that DNA immunisation of mice with plasmids expressing the 28 kDa GST from *S. mansoni* induced both humoral and cell-mediated immune responses against *S. mansoni* 28 kDa GST [45]. Plasmid DNA encoding a 20.8 kDa tegumental antigen expressed on *S. mansoni* sporocysts and adult worms has also been used to immunise mice [46]. Following immunisation with DNA encoding the 20.8 kDa tegumental antigen and subsequent challenge with infectious cercariae, a 30% reduction in worm burden was observed compared with control immunised and challenged mice. Individual plasmid DNA encoding the *S. mansoni* glutathione peroxidase (GPX), the cytosolic superoxide dismutase (SOD) (C-SOD), and the signal peptide containing SOD (SP-SOD) genes have been used to immunise groups of mice [47]. Following DNA immunisation and infectious challenge, worm burden reductions were 55%, 61%, and 45% for the GPX, C-SOD, and SP-SOD DNA immunised groups of mice, respectively, when compared with plasmid DNA immunised controls. This study suggests that plasmid DNA vaccination may afford some immunity against an experimental *schistosome* challenge in an animal model system.

Leishmaniasis is a parasitic disease that occurs in most parts of the world. An incidence of approximately 400 000 new cases per year has been reported and the worldwide prevalence of the disease is thought to be about 12 million cases. Leishmaniasis is caused by several species of intracellular protozoan parasites found in the genus *Leishmania*. There are three main categories of leishmaniasis: cutaneous, mucocutaneous, and visceral leishmaniasis. One of the early successes of genetic immunisation was protection of mice against leishmaniasis by using a DNA plasmid containing the CMV promoter and encoding the major surface glycoprotein, gp63, from *Leishmania major* [48]. *Leishmania major* is responsible primarily for the cutaneous form of leishmaniasis; however, in some cases infection can lead to the visceral form of the disease. Immunisation of mice with this plasmid DNA encoding the gp63 induced T_H1 responses based on IL-2 and IFN-γ secretion from T cells obtained from the spleen and lymph nodes of immunised mice. No detectable IL-4 secretion

was observed when the splenocytes and lymph node cells obtained from gp63-DNA immunised mice were cultured with *L. major* antigens in vitro. The immunised mice also developed significant resistance against *L. major* infection when compared with control plasmid immunised mice. Other investigators have also reported the requirement for a T_H1 response to protect from cutaneous leishmaniasis, whereas a T_H2 response is not protective, and that DNA vaccines may be advantageous in this parasitic disease [49]. The T_H1 response to gp63 induced by DNA vaccines from *L. major* was correlated with protection against murine leishmaniasis [50]. The role of skin-derived dendritic cells as professional antigen presenting cells for priming the DNA induced T_H1 response has also been demonstrated. Protective immunity following DNA immunisation with the gene encoding the immunodominant LACK antigen from *L. major* was also reported in murine systems [51]. The control of disease progression and parasite burden in LACK DNA immunised mice was again associated with antigen-specific IFN-γ production and was dependent on IL-12, indicating the need for a T_H1 response. However, depletion of $CD8^+$ T cells at the time of LACK DNA immunisation or experimental infection also abolished protective immunity induced by LACK DNA injection. This suggested a role for $CD8^+$ T cells in this DNA vaccine induced protection to *L. major*. Together, these studies clearly demonstrate the potential of DNA immunisation for generating protective T_H1 responses in a parasitic system where T_H1 $CD4^+$ T cells are required for protective immunity.

Taenia is a genus of parasitic cestodes that infect the intestines of vertebrates, with *Taenia ovis* representing a tapeworm pathogen of sheep. DNA encoding a host protective antigen, designated 45W, from *T. ovis* was used to immunise sheep. DNA immunisation was compared with responses elicited by a recombinant 45W protein and an ovine adenovirus viral vector expressing the 45W antigen [52]. The effect of the route and mode of immunisation with DNA encoding 45W gene on the antibody response was also evaluated in sheep [53]. Sheep received two immunisations of the three different vaccination strategies. Low levels of specific 45W antibodies were induced by either DNA or the recombinant adenovirus vector immunisation [52]. The antibody levels were boosted by a subsequent immunisation with the recombinant 45W protein administered in Quil A as an adjuvant. The anti-45W levels after the recombinant protein boost were comparable with two injections of recombinant protein in adjuvanted. In the case of priming with DNA and/or

Table 1
Systems where DNA vaccines have been evaluated against parasites

Parasite	DNA vaccine	Reference
Plasmodium falciparum	Circumsporozoite protein antigen	[37]
	Multigene plasmid mixture	[39]
	Somatic transgene	[42]
Plasmodium berghei	Circumsporozoite protein antigen	[40]
	Pre-erythrocytic antigen	[41]
Schistosoma mansoni	28 kDa Glutathione *S*-transferase	[44, 45]
	20.8 kDa Tegumental antigen	[46]
	Glutathione peroxidase	[47]
	Superoxide dismutase	[47]
Schistosoma japonicum	Paramyosin	[43]
	Paramyosin fused to 26 kDa glutathione *S*-transferase	[43]
Leishmania major	Surface glycoprotein, gp63	[48, 50]
	Parasite surface antigen, Ag-2	[49]
	LACK protein antigen	[51]
Taenia ovis	Host protective antigen, 45W	[52]

the adenovirus vector and immunisation with the recombinant protein alone, sheep were protected from experimental challenge with *T. ovis*. These studies suggest that either DNA or recombinant adenovirus vectors can prime the immune response in sheep and the prime-boost strategy can elicit protective immunity. Systems where DNA vaccines have been evaluated against parasitic agents are summarised in Table 1.

7. Systems to develop DNA vaccines against animal coccidioses

A number of potential target antigens to develop DNA vaccination strategies against animal coccidioses is provided in Table 2. Studies that relate to the development and testing of DNA vaccination strategies against coccidia parasites are still in their infancy. A recently described study (S. Sugodira, D Buzoni-Gatel, S Iochmann, M Nacri, D Bout. Protection of neonates against cryptosporidiosis after genetic vaccination of dams. In: Proceedings Vaccines against Animal Coccidioses. COST 820, 1998; Annual Workshop, p 25) employed DNA immunisation to evaluate the vaccination potential against *Cryptosporidium parvum* in goats. *Cryptosporidium parvum* is an intracellular parasite that infects the epithelial lining of cells in the microvilli of the small intestine. This coccidian parasite infects humans and mammals causing cryptosporidiosis, which is an opportunistic infection of immunosuppressed individuals and is often associated with HIV infection and AIDS. Infection of humans is often caused by exposure to oocysts in water or the environment as the result of excretion by infected ruminants. The reduction of infection in the animal reservoir could have a direct impact on the rate of infection in humans. The intranasal genetic immunisation of mice with the gene encoding the *C. parvum* surface sporozoite CP15 antigen generates both humoral and cell-mediated immune responses. Pregnant goats were immunised three times with 200 μg of CP15 encoding plasmid DNA intranasally. The kids were subsequently infected 24 h after birth and monitored over a 3 week period. Nasal inoculation of pregnant goats with DNA encoding the *C. parvum* CP15 gene conferred protection against *C. parvum* in their infants. Kids from DNA vaccinated dams had a lower level of parasite development and their overall growth was not affected. This situation was different from that which was observed in the kids from the unvaccinated mother control groups where higher levels of parasites were obtained and the kids' growth was impeded. Other investigations have described serum and colostrum antibody responses in sheep following DNA injection with a plasmid encoding *C. parvum* CP15 and CP60 surface antigens [54]. The induction of immune responses in mice following DNA immunisation with plasmids encoding antigens from *Toxoplasma gondii* has also been described [55]. These studies provide evidence that DNA vaccination strategies can induce protective immunity in animal coccidioses systems.

Table 2
Potential target antigens to develop DNA vaccines against animal coccidioses

System	Putative antigen targets
Cryptosporidium parvum	Surface sporozoite, CP15 and CP60 antigens Sporozoite antigen, p23
Eimeria tenella	Microneme protein, Etmic 2 Heat shock protein, HSP 70 Merozoite surface antigens
Neospora caninum	Recombinant (rec) proteins, NC-p36 and NC-p43
Toxoplasma gondii	Surface antigens, SAG1 (p30), SAG3 (p43), and SAG4 (p18) Bradyzoite specific heat shock protein antigens Merozoite surface antigen, RAP6
Sarcocystis spp.	Surface sporocyst antigens

8. Human clinical trials with DNA vaccines

There are presently a number of ongoing clinical human trials that are evaluating the effectiveness of DNA vaccination for both prophylaxis and therapy. These trials include HIV, HSV, influenza, malaria, and cancers (Table 3). The

Table 3
DNA vaccination strategies in human clinical trials

Organism/system	Plasmid DNA employed	Utilisation
HIV-1	HIV-1 gp160	Therapeutic
HIV-1	HIV-1 gag/pol	Therapeutic
HIV-1	HIV-1 gp160	Prophylactic
Malaria	Malaria CSP	Prophylactic
HSV-2	HSV-2 gD	Therapeutic
Influenza	Influenza NP	Prophylactic
Hepatitis B	Hepatitis B surface antigen	Prophylactic
Cutaneous T cell leukemia	TCR idiotype	Therapeutic
Carcinoma	CEA	Therapeutic

first DNA based vaccine trial was reported for therapy of HIV infection [56]. A DNA construct expressing the *env* and *rev* genes of HIV-1 was used to immunise 15 asymptomatic HIV infected individuals who were not using antiviral drugs and who had normal levels of $CD4^+$ lymphocytes. Treatment groups received three doses of vaccine (30, 100, 300 μg) at 10 week intervals in a dose-escalation trial. The DNA vaccine was safe and well-tolerated. Antibody responses to HIV gp120 increased among individuals given the higher doses of DNA. Additionally, some increase in CTL activity against gp160 expressing target cells and lymphoproliferative activity was observed.

More recently, a human clinical trial examined a *Plasmodium* derived DNA prophylaxis [57]. Twenty healthy, malaria naive individuals were randomised into four groups of five individuals, and given three injections of 20, 100, 500, and 2500 μg of plasmid DNA encoding the *P. falciparum* CSP at 4 week intervals. These individuals elicited antigen-specific, genetically restricted $CD8^+$ CTL responses as the result of DNA vaccination. This represented the first study where healthy naive humans generated $CD8^+$ CTL responses as the result of DNA immunisation. These two studies are the first published reports describing human clinical trials that evaluated DNA immunisation and they demonstrate that DNA immunisation is capable of generating and boosting the immune response to specific antigens associated with human pathogenic organisms.

9. Issues related to DNA vaccines

DNA vaccines afford a number of advantages and possible disadvantages when compared with alternative vaccination strategies (reviewed in [58] and [59]). The advantages of DNA vaccines include the fact that they can encode multiple immunogenic epitopes and evoke both humoral and cell-mediated immune responses. The immunogenic epitopes are presented to the immune system in their native form. Thus, DNA exhibits the advantages of attenuated vaccines without the safety problems associated with in vivo replication and possible reversion to a virulent form. Plasmid vectors can be rapidly constructed and easily tested. Large-scale manufacturing procedures are available and the DNA can be easily and inexpensively purified to homogeneity, resulting in lower costs to develop and manufacture this type of vaccine. This makes this strategy applicable as a human vaccine approach in underdeveloped countries and as a veterinary vaccine strategy where the cost per dose is of major economic concern. DNA is more thermostable than vaccine strategies which require a cold chain for storage. It should exhibit a longer shelf-life because of the improved stability. The production of combination vaccines employing DNA is also simplified. DNA also allows a more simplified and effective quality control process that provides additional cost benefits.

Some concerns and potential disadvantages of DNA vaccines also exist. These include the potential for integration of the DNA into the host chromosome. Plasmid DNA based vectors often contain nucleic acid sequences from oncogenic viruses, and the possibility for chromosomal integration exists. A second concern of DNA vaccination is the possibility of generating antibodies to DNA. Immune responses to DNA occur in autoimmune diseases, such as systemic lupus erythematosus, and the potential exists that bacterial DNA injection could induce an immune response that might cross-react with host DNA. It

has been reported that antibodies to DNA have been observed following immunisation of mice with bacterial DNA [59, 60]. However, this still represents a theoretical possibility that will require more attention. A third concern is the effect that long-term expression of injected DNA into muscle cells may have on immune responses to subsequent vaccination with different DNA, and whether the immune responses to protective epitopes associated with this second immunisation will be compromised. The unwarranted effects of "original antigenic sin" may come into play in this scenario. The fourth disadvantage is that DNA vaccination strategies are unsuccessful when evaluating non-protein based antigens, such as bacterial polysaccharides and lipids. Yet DNA immunisation represents a promising and new approach to vaccine development that is worthy of evaluation. It remains to be determined how successful DNA vaccines will ultimately become in the future, and how widespread their prophylactic and therapeutic applications will be in the areas of infectious diseases and cancer. Based on present studies in human clinical trials and the multiple successes in a variety of animal models, it appears that DNA immunisation will not fall by the wayside of other vaccination strategies that failed to live up to their initial promise touted during the basic research phase of their evaluation.

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